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Polyphenols composition of wine and grape sub-products and potential effects on chronic diseases

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Abstract. Grape (*Vitis vinifera*) is one of the most cultivated fruit crops in the world, with an approximate annual production of ∼64 million metric tons in 2010 (OIV, 2011). Grapes composition in polyphenols and their extractability which is far from complete and typically reaching only 30–40% depend on grape varieties, vineyard location and the technological parameters during wine making process including destemming, crushing, maceration and pressing. Therefore, grape pomace potentially constitutes a very abundant and relatively inexpensive source of a wide range of polyphenols including monomeric and oligomeric flavan-3-ols (proanthocyanidins) as well as anthocyanins (glucosides, acetylated glucosides and coumarylic glucosides). Moreover, it has been evaluated as a potential source of antioxidant polyphenols which could be used as nutraceuticals or food additives. Actually, phenolic compounds are known to have some health benefits such as a chemopreventive role toward cardiovascular, cancer, and degenerative diseases.

In order to valorize wine by-products from Rhone Valley area, grape and pomace seeds and skins from red wine cultivars at maturity from the vintage 2009 and 2010 (Grenache, Syrah, Carignan, Mourvédre, Counoise and Alicante) have been characterised for their phenolic contents (total phenol contents, tannin and anthocyanin contents - total and individuals; quantification of monomeric and oligomeric proanthocyanidins as well as some anthocyanins (glucosides, acetylated glucosides and coumarylic glucosides)). Ratio of initial phenolic compounds from grape to pomace was also estimated.

The comparison of several wine industry by-products with their respective grapes provided evidence that grape seed and skin pomace extracts still contained appreciable amounts of flavanol-3-ols and anthocyanins even after the fermentation process. Quantitative and qualitative distribution of polyphenols in grape pomaces showed significant differences through varieties. Seed extracts from grapes and pomaces contain exceptionally high amounts of total polyphenols than skin's extracts. This study evidenced seeds from Grenache, Syrah and Alicante and skins from Syrah, Carignan and Alicante as the most interesting fractions to be valorized because of their richest contents in polyphenols compared to the other assessed fractions and varieties. This work further supports that grape pomaces obtained after vinification still retained a significant amount of polyphenols of which level depends largely on the vintage. The use of this by-product would constitute a promising natural source of available polyphenols which could be included in nutraceutical formulation. Activity effects of grape by-products on a chronic disease model with hypertension are given.

1. Introduction

Hypertension is the most important of cardiovascular risk factor worldwide. According to the World Health Organization 2013 data, hypertension accounted for approximately 9.4 million deaths a year,

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contributing to 45% of deaths due to heart disease and 51% of deaths due to stroke. The prevalence of hypertensive people is low under 25 years old but steadily increases to attend 40 % at 65 years old and 90% at 85 years old. It is estimated that by 2030, more than 23 million people will die annually from cardiovascular diseases (CVDs). The importance of oxidative stress, vascular inflammation and endothelial dysfunction has to be highlighted in the development of CVDs. The knowledge of the process has provided new perspectives to elaborate novel pharmaceutical or dietary strategies to control the development of vascular diseases.

Epidemiological studies have shown an inverse correlation between the consumption of polyphenols enriched diet and reduced risks of CVDs [1–3]. The potential mechanisms of preventing CVDs could be related to the antioxidant activity conferred by polyphenols involving different processes and also different compounds. Indeed, more than 8000 phenolic structures can be found. Originating from the shikimic pathway and sharing at least one aromatic ring structure with one or more hydroxyl groups, a large number of polyphenol classes exist. Flavonoids are the major constituents with more than 4000 compounds. They share a common flavan core consisting of 2 aromatic rings (A and B) bounded by 3 carbon atoms which form an oxygenated heterocycle (ring C). This class can be divided into flavanols (catechin, epicatechin), flavonols (quercetin, myricetin, kaempferol), anthocyanins (cyanidin, malvidin), flavones (apigenin), flavanones (naringenin, hesperitin) and chalcones (phloretin). The non-flavonoid group includes compounds such as stilbens (resveratrol) and phenolic acids (gallic acid) [4].

Due to the great diversity of polyphenols, the structure-activity relationship, bioavailability and therapeutic efficacy of the antioxidants differ extensively. To date, grape and wine polyphenols have showed to exert beneficial effects on health [5–7]. For instance, polyphenolic compound in grapes are known to lower oxidative stress [8], to modulate the inflammatory cascade [9, 10], to reduce the oxidation of LDL-c [11, 12] and to induce protection against atherothrombotic episodes including myocardial ischemia and inhibition of platelet aggregation [13, 14]. Most of these health effects have been ascribed to polyphenolic compounds serving as reducing agents in many biological systems by donating hydrogen, quenching singlet oxygen, acting as chelators and by trapping free radicals. Moreover, these antioxidant activities help to limit oxidation of nucleic acids, proteins, lipids, which may initiate degenerative diseases such as cancer, heart disease, dermal disorders and aging [15, 16].

Moreover, polyphenols contribute to the prevention of high blood pressure and endothelial dysfunctions by preventing the NADPH oxidase vascular-dependant oxidative stress and the formation of vasoconstrictors. Polyphenols have been shown, on the one hand to increase the formation of endothelium-derived relaxing factor such as the nitric oxide (NO) [17–19], the endothelium-derived hyperpolarizing factor (EDHF) [20, 21] and the prostacyclin [22, 23] through the redox sensitive PI3-Kinase/Akt pathway and on the other hand, to inhibit the synthesis of contracting factor such as endothelin-1 [24].

A large number of publications evidenced the abundance quantity of polyphenols in grape seeds and skins, showing significant antioxidant capacity. It is therefore obvious that grape derived products such as wines, grape juices and eventually pomaces are natural sources of polyphenols. Grape pomace represents an important under used residue of the wine making process. The dry grape by-product consists of pressed skins, seeds and stems and accounts for about 20% of grapes weight used to make wine [25, 26]. The polyphenol content of grapes and the extraction of grape polyphenols during vinification, which is far from complete, typically reaching only ca. 30–40%, depending on grape varieties, vineyard location and technological parameters of wine making including destemming, crushing, maceration and pressing [27, 28]. Thus, grape pomace potentially constitutes a very abundant and relatively inexpensive source of a wide range of polyphenols including monomeric and oligomeric proanthocyanidins and a diversity of anthocyanin glucosides [29–33].

France is the second wine producer in the world after Italy [34] and among numerous French wine appellations, after Bordeaux, the Rhône valley is the second largest in term of surface (73,468 hectares) and production (2.83 million hL). Vineyards in the Rhône valley grow Mediterranean grape cultivars, such as Grenache, which accounts for 65% of the planted area, as well as Syrah (15%), Carignan (15%) and Mourvèdre (3%) [35]. In order to valorize wine by-products from Rhone Valley area, grape and pomace seeds and skins from red wine cultivars at maturity from the vintage 2009 and 2010 (Grenache, Syrah, Carignan, Mourvèdre, Counoise and Alicante)

have been characterized for their phenolic contents (total phenol contents, tannin and anthocyanin contents, monomeric and oligomeric proanthocyanidins and anthocyanins (glucosides, acetylated glucosides and coumarylic glucosides)). Ratio of initial phenolic compounds from grape to pomace was also estimated. Activity effects of grapes by-products on a chronic disease model with hypertension are given.

2. Materials and methods

This study was conducted in 2010 grapes at maturity and their respective grape pomaces from *V.vinifera* L. cv. Grenache (from two different locations [GRE1 and GRE2]), Syrah (from two different locations [SYR1 and SYR2]), Carignan (CAR), Mourvèdre (MOU), Counoise (COU) and Alicante (ALI), provided from the Rhône Valley area, appellation Châteauneuf-du-Pape. Skins and seeds were removed by hand, lyophilized, grounded then extracted and separated into organic and aqueous fractions using solid-liquid extraction followed by liquid-liquid extraction.

Total polyphenol content (TPC), tannin and anthocyanin contents were determined from skin and seed extracts of grapes and pomaces by the Folin-Ciocalteu, the Bate-Smith and the sodium bisulfite decoloration procedures, respectively. Moreover the proanthocyanidin monomers and oligomers were identified and quantified by HPLC-UV-Fluo [36]. The anthocyanic composition (glucosides, acetyl glucosides and coumaroyl glucosides) in grape and pomace skin extracts were determined by HPLC-UV-MS.

To determine the *in vivo* effect of grape pomace extracts and their potential effect on hypertension, a first experiment were conducted with male 9-week-old spontaneous hypertensive rats (SHR) and their normotensive control Wistar-Kyoto (WKY) obtained from Janvier laboratory (Le Genest St. Isle, France). All rats were maintained at a constant temperature (23°C), with a 12-h dark/light cycle. Water and standard diet were given *ad libitum*. They were daily treated with grape pomace extract (extracted with hydro-alcoholic 70 % solution) by gavage during 6 weeks at a dose of 21 mg/kg/day. Rats were divided in different groups: control group (6 WKY), SHR control group (5 SHR treated with 3% EtOH) and 6 groups of 4 SHR rats treated with pomace extract dissolved in 3% EtOH (SHR1: Grenache seed pomace extract, SHR2: Syrah seed pomace extract, SHR3: Syrah skin pomace extract, SHR4: Carignan seed pomace extract, SHR5: Mourvédre skin pomace extract and SHR6: Alicante skin pomace extract). Blood pressure was measured by the tail-cuff method [37]. The average of three pressure readings was recorded for each measurement.

A second experiment was performed to study the bioavailability of three different extracts using the same experimental design. SHR rats were divided in four different groups: SHR control group (6 SHR

Table 1
Phenolic composition in grape and pomace seed extracts

		TPC	Total tannins	Σ Monomers	Σ Dimers	Trimer
GRE1[a]	Grapes	88.71 ± 0.99	167.82 ± 0.86	2.63 ± 0.09	1.22 ± 0.07	0.23 ± 0.04
	Pomaces	40.47 ± 1.10	83.12 ± 0.03	2.37 ± 0.01	0.67 ± 0.05	0.13 ± 0.01
GRE2[a]	Grapes	58.58 ± 0.201	136.84 ± 5.22	4.31 ± 0.16	1.65 ± 0.06	0.29 ± 0.04
	Pomaces	34.94 ± 0.21	74.88 ± 1.12	3.02 ± 0.07	0.57 ± 0.06	0.11 ± 0.02
SYR1[a]	Grapes	72.84 ± 0.694	123.32 ± 1.36	7.76 ± 0.22	1.25 ± 0.07	0.29 ± 0.01
	Pomaces	35.60 ± 1.77	79.23 ± 1.40	5.38 ± 0.14	0.26 ± 0.02	0.05 ± 0.01
SYR2[a]	Grapes	65.58 ± 0.19	115.64 ± 1.29	5.31 ± 0.12	1.10 ± 0.09	0.28 ± 0.01
	Pomaces	33.01 ± 1.413	68.89 ± 2.26	3.01 ± 0.08	0.54 ± 0.08	0.14 ± 0.02
CAR[a]	Grapes	58.63 ± 3.728	131.73 ± 1.43	4.63 ± 0.52	0.88 ± 0.08	0.19 ± 0.04
	Pomaces	38.79 ± 0.29	78.7 ± 0.21	1.28 ± 0.08	0.46 ± 0.02	0.11 ± 0.01
MOU[a]	Grapes	59.56 ± 1.52	154.91 ± 5.24	3.49 ± 0.03	0.59 ± 0.01	0.20 ± 0.00
	Pomaces	34.52 ± 0.11	69.44 ± 3.05	0.99 ± 0.04	0.39 ± 0.02	0.10 ± 0.01
COU[a]	Grapes	83.45 ± 15.01	143.10 ± 21.91	7.87 ± 0.56	1.37 ± 0.05	0.34 ± 0.03
	Pomaces	40.78 ± 3.09	70.92 ± 4.41	2.84 ± 0.09	0.65 ± 0.05	0.17 ± 0.01
ALI[a]	Grapes	76.42 ± 8.03	148.38 ± 7.26	7.36 ± 0.29	2.22 ± 0.06	0.34 ± 0.03
	Pomaces	44.53 ± 0.36	84.92 ± 3.97	6.51 ± 0.92	1.37 ± 0.08	0.32 ± 0.03

[a]GRE1 and GRE2, Grenache; SYR1 and SYR2, Syrah; CAR, Carignan; MOU, Mourvédre, COU, Counoise; ALI, Alicante. In units of mg/g DW seed. Data are expressed as the mean of triplicate ± standard deviation. TPC, total phenol content; Σ Monomers, sum of catechin and epicatechin; Σ Dimers, sum of B1, B2, B3 and B4; Trimer, C1.

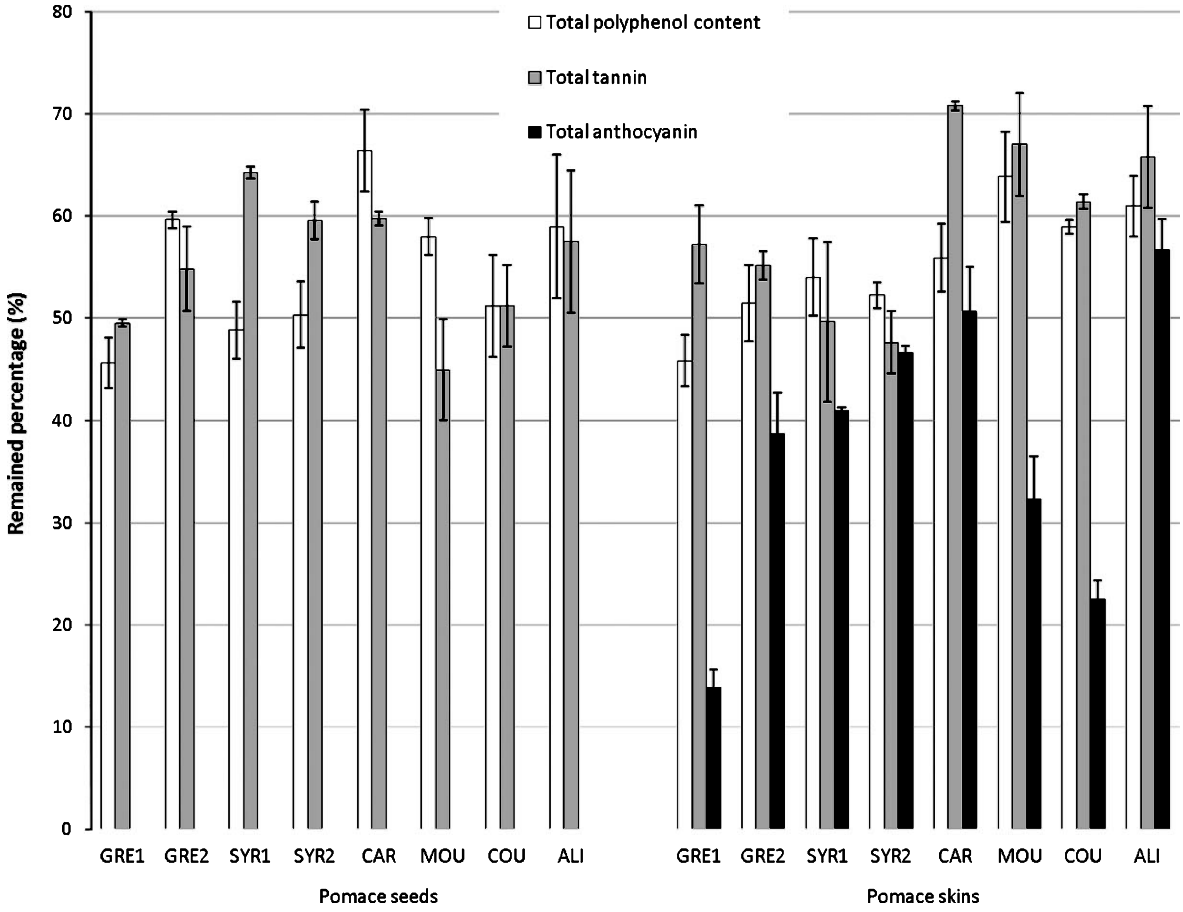

Fig. 1. Remained potential in grape pomace seed and skin extracts in 2010.

treated with 3% EtOH) and 3 groups of 6 SHR rats treated with grape pomace extract at a concentration of 21 mg/kg/day (E1: Grenache seed pomace extract, E5: Mourvédre skin pomace extract and E6: Alicante skin pomace extract). SHR were treated everyday during one week. Urines and faeces were collected at day 0 and day 7 of the experiment at two time point (0–8 h and 8–24 h). Urines and faeces were purified, derivatized and analysed by GC-MC for phenolic acids.

3. Results and discussion

3.1. Grape pomace seeds and skins characterisation

3.1.1. Grape and pomace seed extracts

Total polyphenol content was determined from skin and seed extracts of grapes and pomaces by using the Folin-Ciocalteu methodology. Generally, total polyphenol content in seed extract is higher than in skin extract for grapes and pomaces. The highest levels of TPC were founded in GRE1 and COU (88.7 and 83.4 mg GAE/g DW respectively) while GRE2, MOU and CAR contained lowest amounts with an average of 59 mg GAE/g DW. Total tannin levels ranged from 115.6 mg/g DW in SYR2 to 167.8 mg/g DW in GRE1. After vinification the variability was smaller ranging from 33.0 to 44.5 mg GAE/g DW for TPC and 68.9 to 84.9 mg/g DW for total tannins (Table 1). With all the varieties, more than 45% of TCPs remained in the pomace (Fig. 1).

Concerning proanthocyanidin characterization, flavan-3-ol monomers [(+)-catechin, (−)-epicatechin] and oligomers (B1, B2, B3, B4 and the trimer C1) were identified and quantified. In grapes, COU contained the highest amount of monomeric and oligomeric proanthocyanidins whereas CAR and MOU had the lowest. SYR1 contained a particular rich level of

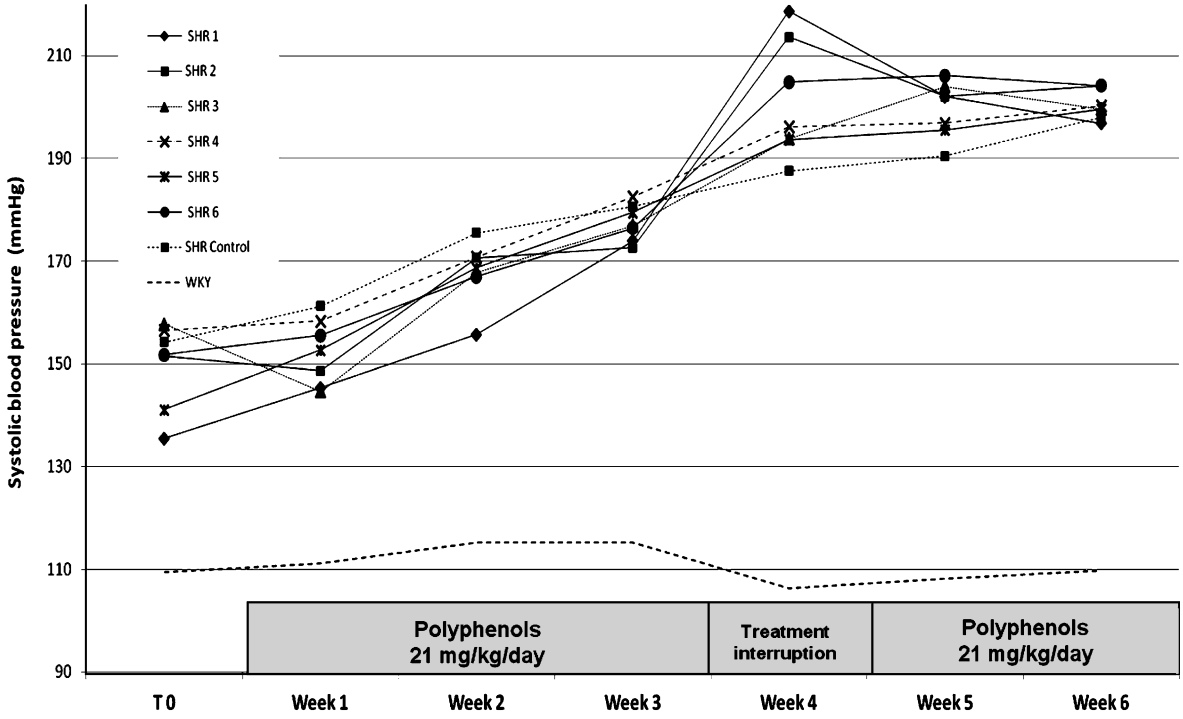

Fig. 2. Effect of polyphenolic extracts on the mean systolic blood pressure during the 6-week study.

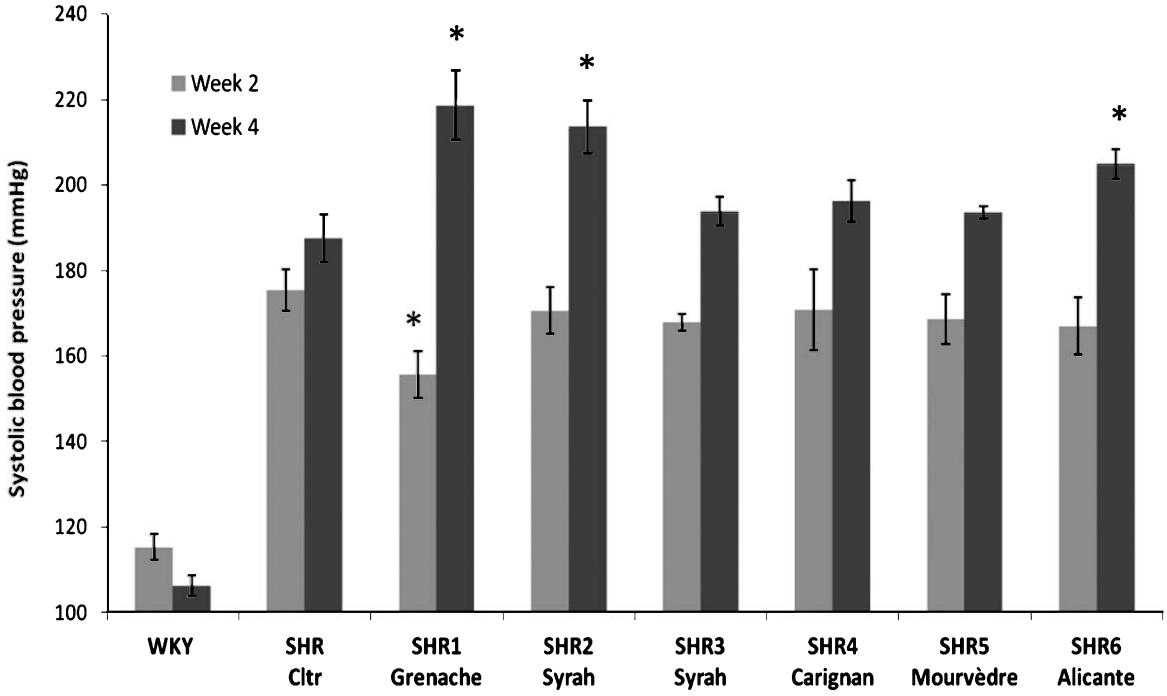

Fig. 3. Systolic blood pressure measured at week 2 (after 14 days of treatment with pomace extracts) and at week 4 (after 7 days of therapeutic interruption) (*$p < 0.05$).

Table 2
Phenolic composition in grape and pomace skin extracts

		TPC	Total tannins	Total antho	Σ Monomers	Σ Dimers
GRE1[a]	Grapes	37.42 ± 0.70	59.52 ± 5.16	11.19 ± 0.18	1.57 ± 0.01	0.07 ± 0.00
	Pomaces	17.14 ± 0.35	33.92 ± 1.36	1.55 ± 0.12	0.51 ± 0.00	0.07 ± 0.00
GRE2	Grapes	37.94 ± 0.05	63.80 ± 0.71	8.44 ± 0.70	3.19 ± 0.00	0.07 ± 0.00
	Pomaces	19.54 ± 1.03	35.18 ± 0.23	3.24 ± 0.03	0.76 ± 0.07	0.08 ± 0.00
SYR1	Grapes	45.17 ± 2.22	73.01 ± 6.02	12.06 ± 0.29	1.84 ± 0.00	0.04 ± 0.00
	Pomaces	24.32 ± 0.00	35.91 ± 1.07	4.94 ± 0.09	0.65 ± 0.04	0.15 ± 0.01
SYR2	Grapes	39.74 ± 0.32	66.83 ± 3.30	10.77 ± 0.12	2.48 ± 0.11	0.04 ± 0.00
	Pomaces	20.77 ± 0.19	31.77 ± 0.11	5.02 ± 0.11	0.82 ± 0.10	0.04 ± 0.00
	Pomaces	20.77 ± 0.19	31.77 ± 0.11	5.02 ± 0.11	0.82 ± 0.10	0.04 ± 0.00
CAR	Grapes	44.91 ± 0.08	65.19 ± 0.71	15.17 ± 0.00	4.29 ± 0.02	0.11 ± 0.01
	Pomaces	25.12 ± 1.10	46.15 ± 0.30	7.69 ± 0.47	0.67 ± 0.00	0.08 ± 0.01
MOU	Grapes	41.27 ± 1.60	70.78 ± 3.09	11.84 ± 0.29	3.89 ± 0.03	0.12 ± 0.00
	Pomaces	26.30 ± 0.27	47.28 ± 1.34	3.82 ± 0.25	0.52 ± 0.03	0.11 ± 0.00
COU	Grapes	34.78 ± 0.01	61.26 ± 1.06	8.69 ± 0.44	3.56 ± 0.00	0.10 ± 0.01
	Pomaces	20.50 ± 0.18	37.61 ± 0.35	1.98 ± 0.02	1.29 ± 0.13	0.16 ± 0.00
ALI	Grapes	52.31 ± 3.89	85.76 ± 8.35	18.15 ± 2.53	8.69 ± 0.05	0.27 ± 0.00
	Pomaces	31.59 ± 1.72	55.30 ± 5.70	9.97 ± 0.82	2.42 ± 0.19	0.12 ± 0.01

[a]GRE1 and GRE2, Grenache; SYR1 and SYR2, Syrah; CAR, Carignan; MOU, Mourvèdre, COU, Counoise; ALI, Alicante. In units of mg/g DW skin. Data are expressed as the mean of triplicate ± standard deviation. TPC, total phenol content; Total antho, total anthocyanins; S Monomers, sum of catechin and epicatechin; S Dimers, sum of B1, B2, B3 and B4.

Table 3
Anthocyanic composition in grape and pomace skin extracts

		Σ Gly	Σ Ace	Σ Coum
GRE1[a]	Grapes	12.17 ± 0.00	0.61 ± 0.01	1.14 ± 0.00
	Pomaces	2.58 ± 0.01	0.18 ± 0.00	0.37 ± 0.00
GRE2[a]	Grapes	6.39 ± 0.01	0.98 ± 0.30	0.74 ± 0.01
	Pomaces	8.57 ± 0.01	0.19 ± 0.00	0.97 ± 0.00
SYR1[a]	Grapes	10.38 ± 0.20	0.70 ± 0.00	1.97 ± 0.00
	Pomaces	10.54 ± 0.00	0.22 ± 0.00	5.29 ± 0.00
SYR2[a]	Grapes	8.15 ± 0.10	0.63 ± 0.00	1.12 ± 0.00
	Pomaces	8.46 ± 0.00	0.18 ± 0.00	1.15 ± 0.10
CAR[a]	Grapes	14.55 ± 0.01	0.74 ± 0.00	3.11 ± 0.05
	Pomaces	13.74 ± 0.02	0.18 ± 0.00	3.05 ± 0.01
MOU[a]	Grapes	6.78 ± 0.16	2.81 ± 0.03	1.62 ± 0.01
	Pomaces	6.23 ± 0.00	0.17 ± 0.00	0.67 ± 0.01
COU[a]	Grapes	6.38 ± 0.01	0.73 ± 0.011	1.43 ± 0.00
	Pomaces	3.28 ± 0.05	0.20 ± 0.00	0.60 ± 0.00
ALI[a]	Grapes	17.40 ± 0.12	1.57 ± 0.00	2.38 ± 0.01
	Pomaces	14.33 ± 0.00	0.20 ± 0.01	4.07 ± 0.00

[a]GRE1 and GRE2, Grenache; SYR1 and SYR2, Syrah; CAR, Carignan; MOU, Mourvédre, COU, Counoise; ALI, Alicante. In units of mg/g DW seed or skin. Data are expressed as the mean of triplicate ± standard deviation. Σ gly, sum of monoglucoside anthocyanins; Σ Ace, sum of petunidin-3-*O*-acetylmonoglucoside, peonidin-3-*O*-acetylmonoglucoside and malvidin-3-*O*-acetylmonoglucoside; Σ Coum, sum of peonidin-3-(6-*O*-p-coumaroyl)monoglucoside and malvidin-3-(6-*O*-p-coumaroyl)monoglucoside.

monomers (7.8 mg/g DW in 2010) while ALI was a source of an appreciable quantity of proanthocyanidins (7.4 mg/g DW of monomers, 2.2 mg/g DW of dimers and 0.34 mg/g DW of trimer C1). Regarding their respective grape pomace, SYR1 and ALI retained a high concentration of monomers with up to 6.5 mg/g DW remaining in ALI. GRE1, COU and ALI were still relatively rich in dimers. Indeed, 90% of monomers and 55% of dimers remained in GRE1 seed pomace and the respective figures for ALI, were 88% and 62%.

3.1.2. Grape and pomace skin extracts

As expected, grape skins contained a lower concentration of phenolic compounds than in seeds. The TPC ranged from 34.8 mg GAE/g DW in COU to 52.3 mg

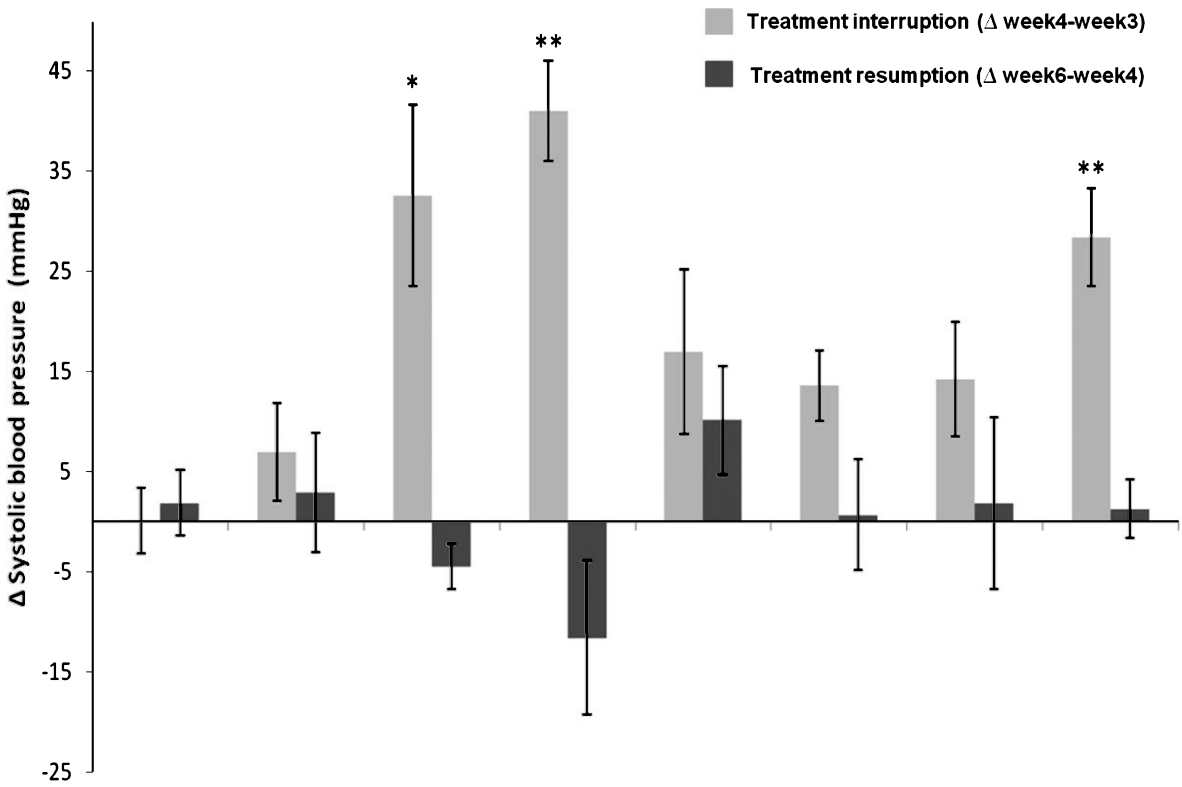

Fig. 4. Variations of systolic blood pressure during interruption and resumption period (*$p < 0.05$, **$p < 0.01$).

GAE/g DW in ALI (Table 2). The highest total tannin levels were found in ALI (85.8 mg/g DW) and for total anthocyanins in SYR1, CAR, MOU and ALI. More than 45% of TPC and total tannins remained in the grape skin pomace of all the varieties. A different trend was observed concerning total anthocyanins especially for GRE1 (Fig. 1) where up to 80% of the initial amounts were extracted. Thus, anthocyanins appeared to be the most easily extractable phenolic compounds during vinification. Indeed, skins are more altered than seeds by the procedures such as pressing, crushing and maceration. During maceration, appreciable substantial quantities of anthocyanins are extracted into wine. As the level of alcohol increases during vinification, anthocyanins are solubilized and released in the acidic matrix [38].

Additional information was obtained when monomeric flavan-3-ols and oligomeric proanthocyanidins were analysed by HPLC. Grape varieties with the highest amounts of monomers and dimers in skins were the CAR, MOU and especially ALI which contained 8.7 mg/g DW of monomers and 0.3 mg/g DW of dimers. The vinification process removed more than 65% of the monomers and especially affected catechin levels (Table 2). Pomace from COU and ALI were the richest in monomeric and oligomeric proanthocyanidins.

The anthocyanin content of skin extracts was analysed by HPLC-UV and the profiles obtained were in good agreement with those obtained in previous studies with *V. vinifera* L. grapes [39, 40] and individual anthocyanin concentrations were well correlated with estimates of total anthocyanin content. For all varieties, malvidin-3-*O*-monoglucoside was the major anthocyanin and accounted for 40% to 55% of total anthocyanins depending on the variety. Grape skin extracts from ALI contained the highest quantities of glucoside-, acetyl- and *p*-coumaroyl-anthocyanins, 17.40, 1.57 and 2.38 mg/g DW, respectively (Table 3). Among "non-teinturier" varieties, SYR1 and CAR were particularly rich in glycosylated and *p*-coumaroylated anthocyanins while MOU was rich in acetylated anthocyanins. Appreciable amounts of anthocyanins remained in grape skin

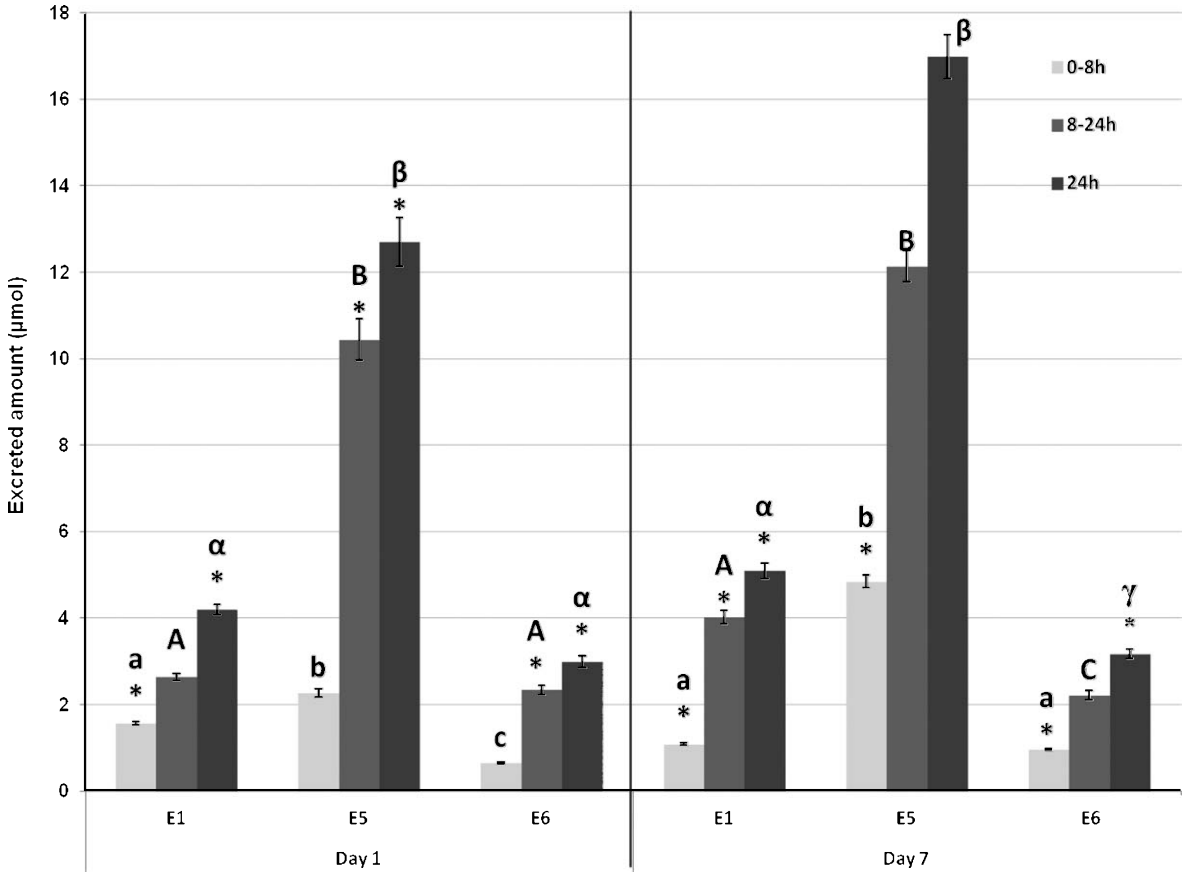

Fig. 5. Total significant urinary catabolites excreted at day 1 and day 7 after ingestion of grape pomace extracts (E1: Grenache seed pomace extract, E5: Mourvèdre skin pomace extract and E6: Alicante skin pomace extract). *Sum of catabolites that are excreted in significantly higher amounts compared to their respective control. a,A,α; Anova was made to compare values obtain during 0–8h, 8–24 and 24 h at day 1 and day 7. Same letters indicate no significant differences between the value ($p < 0.05$). Data are expressed as mean values in µmol ± Std.

pomace of SYR1, CAR and ALI, with up to 14.3 mg/g DW, 0.22 mg/g DW and 5.3 mg/g DW of glucoside-, acetyl- and *p*-coumaroyl-anthocyanins, respectively, being retained. Skin pomace of GRE1 and COU contained the lowest levels of anthocyanins whereas CAR, GRE2, SYR and MOU retained high quantities of glucoside-, acetyl- and *p*-coumaroyl-anthocyanins.

The above data obtained on grape skins and pomace skins indicated that wine making process resulted in a relative increase *p*-coumaroyl derivatives and a decrease of the acetyl-anthocyanins. This phenomenon has also been observed in an earlier study which found that the relative content of *p*-coumaroyl derivatives of malvidin and peonidin was lower in wines than in fresh grape skins but higher in pomace [41]. Slow extraction rates of the *p*-coumaroyl anthocyanins from skins during vinification have been reported, explaining the presence of similar amounts of these anthocyanins in fresh grape skins and pomace skins [42].

4. *In vivo* results

Spontaneously hypertensive rats (SHR) selected for this study is frequently used to carry out studies on the antihypertensive effect of functional food ingredients. This strain represents nowadays the best experimental model for essential hypertension in humans and have shown its efficiency in many studies [43–48].

In order to evaluate the *in vivo* effect of grape pomace extracts and their potential effect on hypertension, rats were fed with different grape pomace extracts at a dose of 21 mg/kg/day, equivalent to a daily dose of

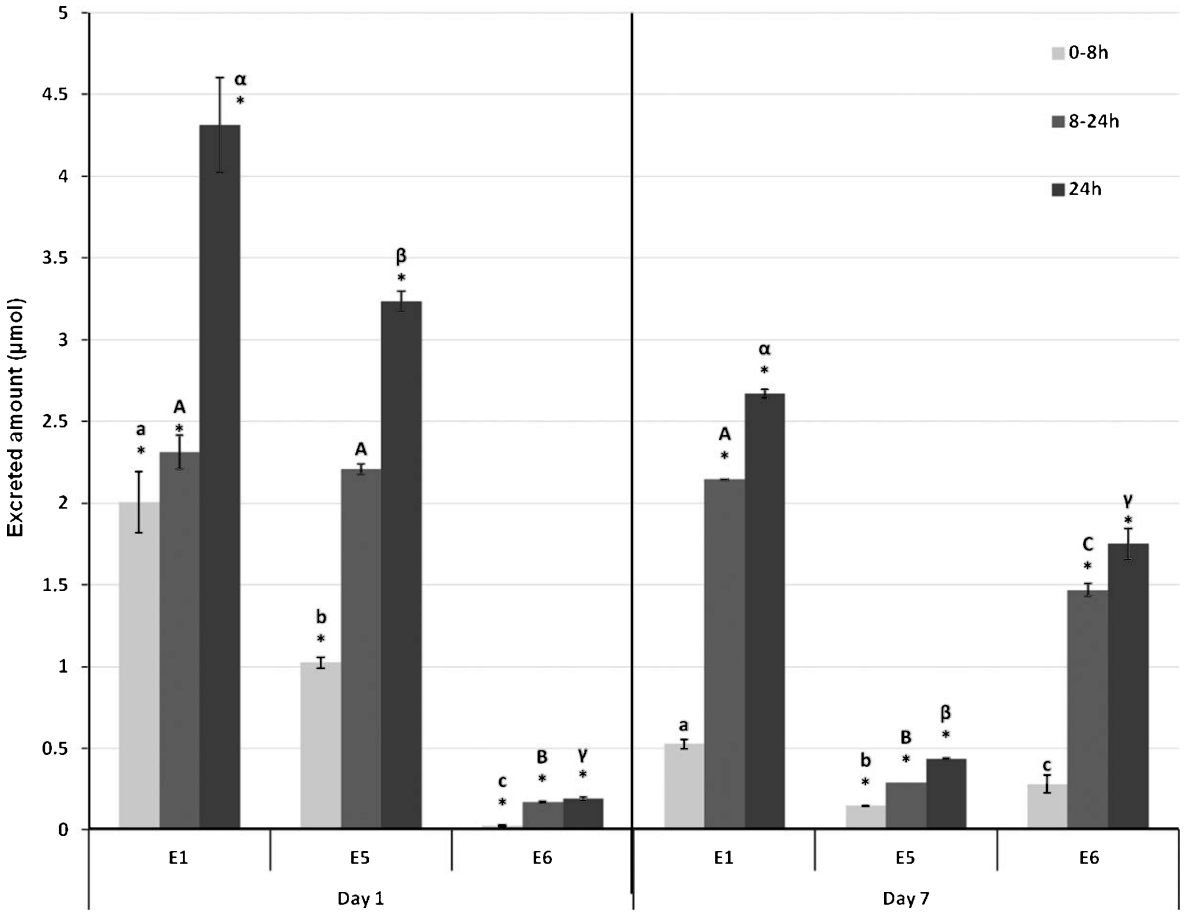

Fig. 6. Total significant faecal catabolites excreted at day 1 and day 7 after ingestion of grape pomace extracts (E1: Grenache seed pomace extract, E5: Mourvédre skin pomace extract and E6: Alicante skin pomace extract). *Sum of catabolites that are excreted in significantly higher amounts compared to their respective control. a,A,α; Anova was made to compare values obtain during 0–8 h, 8–24 and 24 h at day 1 and day7. Same letters indicate no significant differences between the value ($p < 0.05$). Data are expressed as mean values in µmol ± Std.

70 kg human consumption of 0.5 L of wine. Study was conducted during six weeks including three weeks of treatments, one week of treatment resumption followed again by two weeks of treatment.

Concerning the first experiment, as expected, the growth of WKY (3.3 ± 0.09 g/day) were higher than those observed in SHR rats (2.49 ± 0.16 g/day) without significative influence of grape pomace extracts on weight gain. The mean systolic blood pressure of SHR rats was comprised between150 mmHg at the beginning of the experiment and 190 mmHg after five weeks (Fig. 2). Polyphenolic extracts gave to SHR rats have little effect on systolic blood pressure which increased gradually (except for the SHR1 after 2 weeks of treatments) (Fig. 3). However, after three weeks, gavage intolerance were observed and caused difficult the administration of polyphenolic extracts, forcing the interruption of the treatment during one week. This treatment interruption was followed by an increase of the systolic blood pressure in SHR1 (Grenache seed pomace extract), SHR2 (Syrah seed pomace extract) and SHR6 (Alicante skin pomace extract) group compared to the SHR control group. This phenomenon can be intepretated as a « rebound effect » commonly observed with anti-hypertensive drugs and may reveal an antihypertensive effect of grape pomace extracts (Fig. 4). However, the treatment resumption at week 5 and 6 was not followed by a decrease of the systolic blood pressure nor another « rebound effect » during the re-interruption of the treatment (data not shown).

The previous experiment evidenced Grenache seed (E1) and Alicante skin (E6) extracts as having an anti-

Table 4
GC-MS identification of phenolic acids detected in urine following ingestion of seed or skin grape pomace extracts

Rt (min)	Compounds	Target ion *(m/z)*	Qualifier ion *(m/z)*
6.19	Benzoic acid	179	105, 135, 77, 147
6.72	Pyrocatechol	73	254, 239
9.73	3-Hydroxybenzoic acid	267	73, 147, 193, 223, 282, 253
10.03	3-(Phenyl) lactic acid	193	147 73, 220, 267, 295
10.49	3′-Hydroxyphenylacetic acid	73	164, 147, 296, 281
10.91	4-Hydroxybenzoic acid	267	223, 193, 73, 282
11.14	4′-Hydroxyphenyl acetic acid	73	179, 164. 252, 281, 296
12.98	3′, 4′-Dimethoxyphenylacetic acid	209	73, 268, 151, 253
13.28	3-(3′-Hydroxyphenyl)propionic acid	205	192, 310, 177,73
14.16	3′, 4′-Dihydroxyphenylpropionic acid	179	73, 192, 174, 310
14.28	3-Methoxy-4-hydroxybenzoic acid	297	267, 312, 73, 223, 253, 282, 126, 193
14.46	4′-Hydroxy-3′-methoxyphenylacetic acid	73	326, 209, 311, 179, 267, 147
14.76	4′-Hydroxymandelic acid	267	73, 147, 341
15.55	Hippuric acid	105	206, 73
18.65	3-(3′-Methoxy-4′-hydroxy)phenylpropionic acid	209	340, 73, 192, 79, 310, 325
20.41	*p*-Coumaric acid	219	293, 308, 73, 249
21.91	Gallic acid	281	398, 293, 73
27.11	Ferulic acid	338	309, 323, 247, 73, 293
29.47	Caffeic acid	396	219, 291, 73, 307, 381
29.70	3′-Hydroxy hippuric acid	294	73, 193, 147
33.37	Sinapic acid	193	294, 73, 147

Table 5
GC-MS identification of phenolic acids detected in faeces following ingestion of seed or skin grape pomace extracts

Rt (min)	Compounds	Target ion *(m/z)*	Qualifier ion *(m/z)*
6.22	Benzoic acid	179	105, 77, 135, 147, 194
6.60	Phenylacetic acid	73	147, 164, 193, 91
7.80	3′-Phenylpropionic acid	104	75, 147, 207, 179, 222, 91
10.09	3-(Phenyl)-lactic acid	193	73, 147, 220, 295
10.56	3′-Hydroxyphenylacetic acid	73	147, 164, 281, 296
10.88	4-Hydroxybenzoic acid	267	147, 223, 193, 282
11.21	4′-Hydroxyphenylacetic acid	73	179, 164, 296, 281, 252, 147
13.38	3-(3′-Hydroxyphenyl)propionic acid	205	192, 310, 177, 73
18.60	3-(3′-Methoxy-4′-hydroxy)phenylpropionic acid	340	209, 192, 73, 310. 179, 222
27.28	Ferulic acid	338	249, 323, 308, 293, 73, 147

hypertensive potential. In a second experiment, for the study of bioavailability, these two extracts and Mourvédre skin extract (E5) were chosen. Urines and faeces at day 0 and day 7 were collected at 0–8 h and 8–24 h after ingestion of different grape pomace extracts. The phenolic acids of the urine and faecal samples were analysed quantitatively by GC-MS representing urinary and faecal excretion of these compounds in their unconjugated form. Only catabolites that are excreted in significantly higher amounts than the control were taken into account in order to exclude the background noise.

Concerning the urines, a total of 21 of phenolic acids were identified (Table 4). The great majority of the phenolic acids were excreted in 8–24 h urine after grape pomace extracts ingestion (Fig. 5). Total significant catabolite excreted values ranged from 2.33 μmol in E6 to 10.43 μmol in E5 and from 2.21 μmol in E6 to 12.13 μmol in E5 at day 1 and day 7 respectively. The maximum was observed in rats fed with E5 whether at day 1 or day 7. For faeces, a total of 10 phenolic acids were identified (Table 5). The highest faecal excretion was detected in rat fed with the extract E1 with a maximum of 4.31 μmol at day 1 and 2.67 μmol at day 7 of total catabolite excreted over 24 h (Fig. 6). The lowest were obtained with E6 (0.19 μmol) at day 1 and E5 (0.44 μmol) at day 7. Considering these results, especially those obtained in urines, a higher

absorption of flavonoids was observed in rats fed with the Mourvèdre skin pomace extract (E5). Extract E1 (Grenache seed) and E6 (Alicante skin) seemed to be less absorbed despite an antihypertensive potential observed in the first experiment. At this stage, we can hypothesise that flavonoids in E1 and E6 compared to E5 might pass more easily the small intestine barrier and appear in the circulatory system predominantly as glucuronide, sulphate and methylated metabolites and join the bloodstream. A great majority in E5 might largely pass in the large intestine. Indeed, even after the absorption in small intestine, substantial quantities of flavonoids pass from the small to the large intestine and are subjected to the action of colonic microflora where most of them are broken down and yield a diversity of phenolic acids [49, 50]. Colonic-derived catabolites can possibly be absorbed into the bloodstream again and passes through the body prior to excretion in urine.

For the meantime, any conclusions can be made. The analysis has to be completed with data obtained from LC-MS which allowed the identification of metabolites in the form of *O*-methylated, sulphated and glucuronidated in plasmas, urines and tissues. Information concerning absorption in the small intestine will then be provided and complete the bioavailability study.

5. Conclusions

This investigation screened the phenolic composition of by-products obtained after vinification of different Mediterranean grape varieties, in order to assess their potential for nutraceutical applications. Despite extraction during vinification, grape seed and skin pomace extracts contained appreciable amounts of flavan-3-ols and anthocyanins. The quantitative and qualitative distribution of polyphenols in grape pomaces showed significant differences through varieties. Seeds from Grenache (GRE1), Syrah (SYR1) and Alicante and skins from Syrah (SYR1), Carignan and Alicante were evidenced as the most interesting fractions because of their richest polyphenol content. The *in vivo* study also showed the efficiency of Grenache seed pomace and Alicante skin pomace extracts to regulate blood pressure which was illustrated by a rebound effect. In this work, the degradation and subsequent excretion of grape pomace polyphenols knows to enter the large intestine after ingestion of grape seed/skin pomace extracts were studied by GC-MS analysis. These results should be completed with data obtain from LC-MS in order to get an overall picture of the bioavailability of each extracts. These first results evidenced grape pomace extracts as useful by-products which could be used as a natural source of polyphenols and antioxidants for nutraceutical formulations.

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